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GROUPS WHICH ARE DECOMPOSABLE INTO TWO NON-INVARIANT CYCLIC SUBGROUPS

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A group G is said to be decomposable into the two subgroups H_1 and H_2 if all of its operators appear in the product H_1H_2 and if neither H_1 nor H_2 is identical with G. If G is decomposable into H_1H_2 it is also decomposable into H_2H_1, since the inverses of all the operators of a group constitute the same group. A necessary and sufficient condition that every operator of G appears only once in such a product when G is thus decomposable is that H_1 and H_2 have only the identity in common. If the cross-cut of H_1 and H_2 is an invariant subgroup of the decomposable group G, then the corresponding quotient group is decomposable into two subgroups which have only the identity in common. In particular, if a group is decomposable into two abelian subgroups its quotient group with respect to the cross-cut of these subgroups is decomposable into two abelian subgroups which have only the identity in common.

In what follows it will be assumed that G is decomposable into the two cyclic non-invariant subgroups H_1 and H_2. In particular, G must be a non-abelian group and its order g must be the product of at least three prime numbers. It should be noted that the cross-cut of two conjugate cyclic groups is invariant under all the operators which transform one of these groups into the other, while this is not necessarily true as regards the cross-cut of two conjugate non-cyclic groups. From this theorem it results directly that when H_1 and H_2 are of the same order then each of them must involve an invariant subgroup of G whose order exceeds the identity; and when their orders are different, then at least the larger one must have this property since the index of the cross-cut of two conjugate subgroups under one of these subgroups is always less than their common index under the entire group.[1] Hence there results the following theorem: *If a group is decomposable into two cyclic subgroups then it contains an invariant cyclic subgroup whose order exceeds one and which gives*

rise to a quotient group which is either cyclic or is also decomposable into two cyclic subgroups.

As a very special result from this theorem it may be noted that every group which is decomposable into two cyclic subgroups is solvable but a solvable group is not necessarily thus decomposable. It may also be observed that the invariant cyclic subgroup noted in this theorem may always be so selected that it is contained in one of the two cyclic subgroups in question. When g is of the form p^m, p being a prime number, it is obvious that $m \geqq 4$ since a subgroup of index p is necessarily invariant under a group of order p^m. We proceed to determine all the possible groups of order p^4 which are decomposable into two non-invariant cyclic subgroups. It is obvious that each of these subgroups is of order p^2 and hence it must involve a subgroup of order p which is invariant under G in accord with the theorem stated above.

Since every invariant subgroup of order p in a group of order p^m is composed of invariant operators it results that the central of G must be of order p^2 and of type (1,1). The commutator subgroup of G must therefore be of order p and it must be one of the $p - 1$ such subgroups of the central which appear neither in H_1 nor in H_2. Hence G contains $p + 1$ abelian subgroups of order p^3 and at least two of them are of type (2,1) since H_1 and H_2 cannot appear in the same such subgroup. Since G is completely determined by these conditions *there is one and only one group of order p^4, p being any prime number, which is decomposable into two non-invariant cyclic subgroups.* When p is odd this group is also decomposable into an invariant and a non-invariant cyclic subgroup of order p^2 but this is not possible when $p = 2$.

When $p = 2$ the group defined in the preceding theorem is conformal with the abelian group of type (2,1,1) and it admits three-fourths isomorphisms. Two of its abelian subgroups of order 8 are of type (2,1) while the third is of type (1,1,1). When $p > 2$ this group is always conformal with the abelian group of type (2,2) and each of its $p + 1$ abelian subgroups of order p^3 is of the type (2,1). One of these is characteristic while the others are conjugate under the holomorph of G. The p^{th} powers of the operators of order p^2 in this characteristic abelian subgroup of order p^3 are commutators of G while this is not the case as regards the other p abelian subgroups of this order. When $p > 2$ this group can be represented as a transitive substitution group in only the regular form while it can also be represented in one and only one way as a transitive group of degree 8 when $p = 2$. In every case G involves one and only one characteristic subgroup of order p, viz., the commutator subgroup, and all the other subgroups of this order are conjugate under the holomorph of G when and only when $p > 2$.

It was noted above that g must be the product of at least three prime

factors, and when there are only three such factors they cannot all be equal to each other. It is easy to see that in this case they could also not all be distinct. Hence it results that when g is the product of just three prime factors it must be of the form pq^2, where p and q are distinct prime numbers. If the order of H_1 were q^2 then it might be assumed that the order of H_2 would be p and that G would involve an invariant subgroup of order q. This is impossible since the corresponding quotient group could not then involve an invariant subgroup of order q. Hence it may be assumed that the order of H_1 is pq and that of H_2 is q, and that G involves an invariant subgroup of order q. As the corresponding quotient group could not involve an invariant subgroup of order p it results that $q - 1$ is divisible by p and that the central of G is the given invariant subgroup of order q.

From the preceding paragraph it results that if a group of order pq^2 is decomposable into two non-invariant cyclic subgroups it must involve as an invariant subgroup the non-cyclic group of order q^2 and p must divide $q - 1$. As an operator of order p must transform the operators of one of the cyclic subgroups of this group of order q^2 into themselves and those of another such subgroup into powers of themselves the automorphism of this invariant non-cyclic subgroup of order q^2 is completely determined and hence there is one and only one such group. That is, *whenever* $q - 1$ *is divisible by* p *there is one and only one group of order* pq^2 *which is decomposable into two non-invariant cyclic subgroups, and there is no such group when* $q - 1$ *is not divisible by* p. This group is also decomposable into an invariant subgroup of order q and a non-invariant cyclic subgroup of order pq, and its $q - 1$ non-invariant subgroups of order q are conjugate in sets of p each. The smallest possible order of such a group is 18, and this group is the direct product of the group of order 3 and the symmetric group of order 6.

When a group is decomposable into two non-invariant cyclic subgroups which have more than the identity in common the quotient group with respect to the cross-cut of these subgroups is decomposable into two non-invariant cyclic subgroups which have only the identity in common. From this theorem it results directly that if the order of a group which is decomposable into two non-invariant cyclic subgroups is the product of three prime numbers then these subgroups have only the identity in common. It is, however, more important to note in this connection that a necessary and sufficient condition that all the operators of a group can be represented in the form $s^\alpha t^\beta$, where s and t are operators of the group, is that the group is decomposable into the cyclic subgroups generated by s and t, respectively. Hence the preceding developments have close contact with very early developments in the theory of finite groups as well as two articles recently published in these PROCEEDINGS, volume 13 (1927),

page 759, and volume 16 (1930), page 400. The theorem noted at the close of the second paragraph seems to be especially useful in dealing with these groups and it should supersede the corresponding theorems relating thereto, published in the articles just cited.

A necessary and sufficient condition that a group is decomposable into two cyclic subgroups is that it contains at least two cyclic subgroups such that the product of their orders divided by the order of their cross-cut is equal to the order of the group. In closing it may be desirable to direct attention to a very elementary infinite system of groups composed of groups which are separately decomposable into two non-invariant cyclic subgroups but not also into two cyclic subgroups of which at least one is invariant. Such a system may be constructed by forming the direct products in succession of two non-abelian groups of orders pq and pr, respectively, where p, q, r are prime numbers and q may or may not be equal to r. All such groups are decomposable into two cyclic groups of orders pq and pr, respectively, and the smallest group which belongs to this system is the holomorph of the symmetric group of order 6. In this case $p = 3$ and $q = r = 2$.

[1] Miller, Blichfeldt, Dickson. *Finite Groups*, **1916**, page 67.

ON THE DUALITY THEOREMS FOR THE BETTI NUMBERS OF TOPOLOGICAL MANIFOLDS

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The terms and notations used throughout this note will be those of Lefschetz.[1] A *topological manifold* M_n is a compact metric space whose defining neighborhoods are n-cells. M_n can be covered by a finite set of n-cells. Any such set is called a *covering set*.

The Betti numbers of M_n have been defined by W. Flexner[2] by means of a nexus of oriented cells, called *elemental cells*, on M_n, which overlap each other. When these cells are small enough the Betti numbers of M_n (absolute, mod m, etc.) can be calculated from the incidence relations of these cells as in the purely combinatorial case. The object of the present note is to announce, and briefly to describe, the proof of the duality theorems for these Betti numbers.

Because more than two elemental n-cells of a covering are in general incident with each elemental $(n - 1)$-cell of M_n, the construction of a dual complex seemed impossible, hence the relations of the p-cycles and

$(n - p)$-cycles depending on the manifold as a whole had to be established in some other way. W. Flexner proved that it was possible to define the Kronecker index $(\gamma^j_{n-p} \cdot \gamma^i_p)$ of cycles γ^j_{n-p}, γ^i_p, on M_n in such a way that if either γ^j_{n-p} or $\gamma^i_p \approx 0$, $(\gamma^j_{n-p} \cdot \gamma^i_p) = 0$. Since M_n has the Urysohn-Menger dimensionality n, it has a homeomorph on an S_r as proved by Menger.[3] Alexander has given an elementary proof for the special case of M_n, which has been embodied in the paper by Flexner already quoted.[2] We identify M_n with its image in S_r, which we call M_n from now on. Taking advantage of the immersion property we show that given a set of h independent cycles γ^i_p, there exist h cycles γ^j_{n-p} such that

$$(\gamma^j_{n-p} \cdot \gamma^i_p) = \delta_{ij}. \tag{1}$$

By Alexander's duality theorem[4] as extended by Alexandroff,[5] to the set of non-bounding p-cycles γ^i_p on M_n there corresponds a set of $(r - p)$-chains, C^j_{r-p}, in S_r, whose boundaries do not cut M_n and such that $(C^j_{r-p} \cdot \gamma^i_p) = \delta_{ij}$.

The intersection of C^j_{r-p} and M_n can be defined as an $(n - p)$-cycle G^j_{n-p} in S_r and arbitrarily close to M_n. Then G^j_{n-p} can be deformed into γ^j_{n-p} on M_n, in such a way that

$$(\gamma^j_{n-p} \cdot \gamma^i_p)_M = (C^j_{r-p} \cdot \gamma^i_p)_S = \delta_{ij}. \tag{2}$$

From this follows that the cycles γ^j_{n-p} are independent, which leads immediately to the duality theorem.

Having given a brief outline of the proof, we will explain some of the steps in more detail. To obtain the intersection of M_n and C^j_{r-p} it is necessary to replace C^j_{r-p} by another chain D^j_{r-p} homologous to C^j_{r-p} in S-M, and at an arbitrarily small distance from it. C^j_{r-p} is such that no cells of C^j_{r-p} of dimensionality $< r - n$ meet M_n. That the construction of D^j_{r-p} is possible follows from the duality theorem of J. W. Alexander.[4] D^j_{r-p} also is made of cells so small that the intersection of each cell of D^j_{r-p} with M_n is entirely contained in an n-cell on M_n. Lefschetz has shown (loc. cit., ch. 4) that the interesction of two arbitrary chains, each of whose boundaries does not meet the other chain, can be approximated by taking the intersection of simplicial chains approximating the arbitrary ones. If the approximations are close enough, all intersections due to various approximations are homologous in a neighborhood of the geometric or point-set intersection.

The intersection G^j_{n-p} of M_n and D^j_{r-p} is built up by taking first the intersection of the $(r - n)$-cells of D^j_{r-p} with one of the n-cells E_n of M_n covering their geometric intersection. Then the intersection of the $(r - n + 1)$-cells of D^j_{r-p} with the E_n covering their geometric intersections with M_n are each taken and modified so that the boundary of

the intersection is the intersection of the boundary with M_n, which was obtained when the $(r - n)$-cells were treated. This process is continued until the $(r - p)$-cells have been treated. Because of the modification at each step to secure a proper boundary for the intersection, the intersection of M_n and D^j_{r-p}, defined as the sum of the intersections of its component cells, is a cycle.

Another device is used to prove that

$$(\gamma^j_{n-p} \cdot \gamma^i_p)_M = (D^j_{r-p} \cdot \gamma^i_p)_S. \tag{3}$$

It amounts to choosing as the h independent non-bounding cycles on M_n a set with special properties. In the first place these particular cycles are composed of pieces each simplicial in some n-cell of M_n. The simplicial pieces are connected by point-sets on M_n, each of which is an arbitrarily close neighborhood of the boundary of a simplicial piece. This means that the non-simplicial parts of the cycles are neighborhoods of sets of dimensionality $p - 1$. The existence of such a cycle homologous to each arbitrary cycle is proved by means of the deformation theorem of Alexander and Veblen. Secondly, these cycles are altered and combined algebraically until they are such that the removal of h of their simplicial n-cells will reduce to zero the pth connectivity of the subset of the points of M_n carrying the independent cycles. This gives a way of forcing the intersection of D^j_{r-p} and γ^i_p into regions which can each be covered by an arbitrarily small n-cell of M_n.

From this point on there are no major difficulties. What happens essentially is that all required intersections are reduced to polyhedral intersections on an E_r of S_r. In that region, for $r > 2n + 1$, a choice always possible, anyone covering E_n of M_n can be indefinitely approximated by a non-singular polyhedral cell which takes the place of E_n, and the intersection theory developed by Lefschetz leads to (3), then to (2) completing the proof of the duality relations.

Once the duality relations are proved we can apply the results obtained by Lefschetz for the coincidences and fixed points of continuous transformations of combinatorial manifolds. In particular we have an immediate definition for Brouwer's *degree* as in the case of absolute manifolds. For, assuming M_n connected, the duality theorem implies that there exists a fundamental n-cycle γ_n on M_n, and the degree of T is merely the coefficient d in the homology $T \cdot \gamma_n \approx d\gamma_n$. See in this regard recent papers by Wilson[6] and Hopf.[7]

In concluding it may be said that the methods here outlined enable us to develop a systematic intersection theory for topological manifolds immersed in one another.

[1] Lefschetz. Topology, The Providence Colloquium Lectures. (Due to appear in the Fall of 1930.)

[2] W. W. Flexner. On Topological Manifolds. In course of publication with the *Annals of Math.* (1930).

[3] Menger. Dimensionstheorie. Berlin (1929).

[4] Alexander. *Trans. Amer. Math. Soc.*, **25** (1922), 333–349.

[5] Alexandroff. *Annals of Math.*, **30** (1929), 101–187.

[6] W. Wilson. *Math. Annalen*, **100** (1928), 552–578.

[7] Hopf. *Math. Annalen*, **100** (1928), 579–608.

RECIPROCAL TRANSLOCATIONS IN DROSOPHILA AND THEIR BEARING ON OENOTHERA CYTOLOGY AND GENETICS

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Belling[1] suggested that the chromosome rings found in Oenothera by Cleland[2] and others are to be explained as resulting from exchanges of ends between non-homologous chromosomes, so that one chromosome of a given complex is homologous at one end to one chromosome of a second complex, and at the other end to a different chromosome of the second complex. Håkansson[3] and Darlington[4] have elaborated this view. In a recent issue of this JOURNAL Cleland and Blakeslee[5] have carried the analysis through in detail, showing that it gives self-consistent results. It enables one to predict the configurations of untried combinations, and is to a certain extent in agreement with the genetic data of Renner[6] and Oehlkers.[7]

We have studied cases in Drosophila that conform to the scheme that is required to fit Oenothera. The details of these experiments are now ready for publication, and will appear elsewhere. We wish here to point out their bearing on Oenothera problems, since it has been possible to carry out a far more detailed and accurate genetic analysis than will be possible in Oenothera for many years.

We have studied four cases of translocations involving the two large *V*-shaped pairs of autosomes (II and III) of Drosophila melanogaster. One case (translocation *E*) apparently arose spontaneously. A section from the end of the left limb of chromosome II became detached, and re-attached near the middle of the left limb of chromosome III. This case, while of interest in other connections, does not furnish a good parallel to Oenothera and need not concern us further here. The other three cases (translocations *A*, *B* and *C*) all arose in x-ray experiments, and are all reciprocal translocations—i.e., they represent an exchange of parts

between chromosomes II and III. *A* and *C* are alike, *B* is of a somewhat different nature; they may be discussed separately.

In *A* and *C* both II and III broke at their mid-points (apices of the *V*'s, spindle-fibre attachment-points), and then the two left limbs became attached to each other, as did the two right limbs. If the normal chromosomes be designated II *L* II *R* (*L* = left limb, *R* = right limb) and III *L* III *R*, the new arrangement is II *L* III *L* and II *R* III *R*. Flies homozygous for the new arrangement do not survive—as is the case for the majority of the translocations found in Drosophila. Their death in the present instance, however, may be shown not to be due to the new arrangement of parts, since flies that receive translocation *A* from one parent and translocation *C* from the other are fully viable.

When flies heterozygous for translocations *A* or *C* and for the normal chromosome complement are mated to normal flies (with sufficient mutant characters present to mark all the chromosome segments concerned) only two types of offspring are produced—homozygous normal (from II *L* II *R*, III *L* III *R* gametes), and flies heterozygous for the translocation (from II *L* III *L*, II *R* III *R* gametes). Matings of translocation heterozygotes together show that four other types of gametes are formed and are functional—II *L* II *R* II *L* III *L*, III *L* III *R* II *R* III *R*, II *L* II *R* II *R* III *R*, and III *L* III *R* II *L* III *L*. These (which give inviable zygotes unless each meets its complementary class at fertilization) are produced in smaller numbers then are the first-named two types. The data indicate that about 60 per cent of all the gametes belong to the II *L* II *R* III *L* III *R* and II *L* III *L* II *R* III *R* classes—a result which checks exactly with the independent observation that, from normal female crossed to heterozygous translocation male, about 60 per cent of the eggs give viable offspring, and about 40 per cent die in early stages. Crossing-over occurs in all regions in females heterozygous for these translocations, but is appreciably less than in normal flies.

The cytological picture shows four *V*-shaped chromosomes, as in normal melanogaster; but the somatic pairing, characteristic of the Diptera, is upset. In no case have we seen two pairs each with both members parallel—as is the commonest arrangement in normal flies. In two figures there was a cross-shaped arrangement, each arm of each chromosome paired with another arm, but these two other arms belonging to different chromosomes. The other figures seen approximated more or less closely to this arrangement, which is the one the genetic results led us to expect. The maturation divisions have not been studied; we do not know whether or not rings of the Oenothera type are present, but they may be expected.

In translocation *B*, chromosome III broke at its mid-point, chromosome II somewhat to the left of its middle, and again the two left portions united as did the two right ones. Here the cytological results show one unusually

long chromosome and one short one, with a disturbance of the somatic pairing. The most important difference in the genetic results is that *B* gives more irregular gametes than do *A* and *C*. The gametes formed are II *L* II *R* III *L* II *L* and III *R* II *R* III *L* III *R*, not the other two types (III *R* II *R* II *L* II *R* and III *L* II *L* III *L* III *R*), since the composite including more than a half of II always passes to the opposite pole from the normal II. In agreement with this result is the fact that only 47 per cent of the eggs from normal females fertilized by males heterozygous for translocation *B* were viable, as opposed to the 60 per cent from *A* and *C*.

In general, translocations *A* and *C* furnish clear models for the Belling scheme, while *B* and *E* do not give results that so closely parallel the behavior of Oenothera. One may infer that the Oenothera translocations represent exchanges of equal portions—very probably halves—of chromosomes. This is in agreement with the observation that there are no appreciable size differences among the chromosomes of Oenothera.

That crossing-over occurs in flies heterozygous for the translocations is consistent, for it is difficult to avoid the conclusion that such Oenothera types as the sulfur-flowered forms of biennis and suaveolens arise through crossing-over within chromosome rings. There are also indications that crossing-over is more frequent in the paired chromosomes of Oenothera than in the rings—just as it is more frequent in normal Drosophila than in heterozygous translocation flies.

That translocations in Drosophila are frequently lethal in homozygous form when they first arise is suggestive in view of the well-known fact that the majority of the Oenothera complexes carry zygote lethals. One may surmise that the lethals and the rearrangements of parts of chromosomes arose simultaneously.

One difference between the cases here described and those in Oenothera is that the irregular types of gametes are functional in Drosophila, whereas in Oenothera they evidently are represented by the empty pollen-grains and ovules. The difference is, obviously, to be referred to the relatively more complex development of the haploid stage in plants than in animals.

Translocations *A* and *C* furnish all the necessary requirements for the Belling interpretation, and it has been possible to analyze them in great detail. We feel confident that the Belling interpretation is essentially correct for Oenothera, both because of this definitely established parallel case and because of the power of prediction that arises from it.

[1] Belling, J., 1927. "The Attachment of Chromosomes at the Reduction Division in Flowering Plants." *J. Genet.*, **18**, 177–205.

[2] Cleland, R. E., 1923. "Chromosome Arrangements during Meiosis in Certain Oenothera." *Am. Nat.*, **57**, 562–566.

[3] Håkansson, A., 1928. "Die Reduktionsteilung in der Samenanlage einiger Oenotheren." *Hereditas*, **11**, 129–181.

[4] Darlington, C. D., 1929. "Ring-Formation in Oenothera and Other Genera." *J. Genet.*, **20**, 345–363.

[5] Cleland, R. E., and A. F. Blakeslee, 1930. "Interaction between Complexes as Evidence for Segmental Interchange in Oenothera." *Proc. Nat. Acad. Sci.*, **16**, 183–189.

[6] Renner, O., 1925. "Untersuchungen über die faktorielle Konstitution einiger komplexheterozygotischen Oenotheren." *Bibliotheca Genetica*, **9**, 1–168.

[7] Oehlkers, F., 1926. "Erblichkeit und Zytologie einiger Kreuzungen mit Oenothera strigosa. (Vererbungsversuche an Oenotheren 4)." *Jahrb. wissensch. Botanik*, **65**, 401–446.

NOTES ON THE PLASTID AND OTHER CYTOPLASMIC BODIES DURING SPOROGENESIS AND SPERMATOGENESIS IN POLYTRICHUM COMMUNE

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The question of the nature, the value and the function of cytoplasmic bodies cannot be settled unless we possess an exact account of the behavior of such bodies throughout the complete life history of a plant form. Because such an account is lacking the author has undertaken to study the stages of development from spore to spore in the mosses *Polytrichum commune* and *Catharinea undulata*. On account of possible alterations due to fixation, careful comparisons are being made between the effects of killing solutions such as Bouin and Flemming, and the more specialized techniques, Regaud, Benda, Kolatchev, and Da Fano. The studies on fixed cells are being controlled as far as possible by studies on living material.

Some points have already been well established by others but many questions remain unsettled. That the chloroplast persists during sporogenesis was demonstrated by the work of Sapěhin. However, little is definitely known of the origin of the archesporial plastid, of its division in the archesporial line; and of its behavior during meiosis and in the development of the spore. The relation of the mitochondria and the plastid during sporogenesis is a second question, which has been rather obscured by the recent work of Senjaninova.[1] No botanist has as yet concerned himself with the development of the plastid or of other cytoplasmic bodies in the archeogonium. Allen's[2] account of spermatogenesis undoubtedly gives as much detail as it was possible to obtain with fixing solutions containing acetic acid. But, in view of the recent work on animal spermatogenesis, there is much in the account of sperm development

in *Polytrichum* that is extremely suggestive for further study. The behavior of the plastid and of the mitochondria during sperm formation has, as yet, not been thoroughly investigated. There rests as well the question of the development of the plastid in the meristematic tissue. Whether or not this last point will still fall into Sharp's category of "an unknown behavior of invisible bodies" remains to be seen. It may be possible to obtain some positive evidence on plastid formation from the spore just before and during germination, for at this stage the number of plastids increases very rapidly. These are the principal questions and, although the study of them and of others is far from completion, some extremely interesting results have been obtained and publication of them seems to be warranted.

I. *The Plastid in Sporogenesis.*—Each cell of the archesporium contains one or two large bodies which appear to be plastids. The first step was to ascertain the true nature of these bodies by tracing their development from pre-archesporial cells. Owing to technical difficulties in making the first preparations of late pre-archesporial stages we have not followed completely the evolution of the large body observed in the archesporial tissue. However, the stages we now possess allow the conclusion that the archesporial body comes from a transformation of the pre-archesporial plastid.

In pre-archesporial tissue the plastids are small and all of practically the same visible structure. The archesporial plastid has a structure differing not only from the pre-archesporial plastid but even from the largest plastids in the other tissues. It no longer gives a positive reaction for starch. It consists essentially of two parts: (*a*) a fairly broad filament which stains heavily in material fixed in silver or osmic acid, and must therefore be composed of a substance of reducing nature; (*b*) to this filament there is attached a thin, flat pellicle possessing the same properties although in a much weaker degree, staining gray rather than black. There seems to be no reason to consider this structure as due to fixation, for although such fluids as Flemming, Bouin and usually Regaud practically destroy the archesporial plastid, in Kolatchev, Da Fano, Benda and Champy-Kull it exhibits a similar structure and is always sharply outlined against the cytoplasm. Moreover, both border and pellicle may be clearly seen in living material.

The two components of the archesporial plastid are so distinct and their behavior so characteristic that the following descriptions will be very much facilitated if simple names are given them. The filament because of its form and properties will be called the *plastonema*, while for the pellicle the combination *plastosome* seems to be appropriate.

The shape of the plastonema and its relation to the plastosome appear clearly in figures 1, 2 and 3. One can see that the plastonema partially

surrounds the plastosome, forming to some extent the edge of the plastosome toward the cell membrane. It is impossible to explain such a structure according to the statements of Owens and Bensley,[3] that osmium and silver impregnate only the edge of vacuoles, for if the plastonema should be only the border of a vacuole its form would be that of a hollow sphere. The plastonema is a true cytoplasmic body.

During the division of the nucleus, at which time the cell attains its greatest dimensions, it contains two plastids located at its poles (Fig. 1). The plastonema lies parallel with the cell membrane, the plastosome extending toward the nucleus and the other plastid. These two plastids in all successful preparations are connected by a fine filament. In the young and smaller daughter cells the plastonema takes the form of a semi-circle or horseshoe partially surrounding the plastosome (Fig. 2).

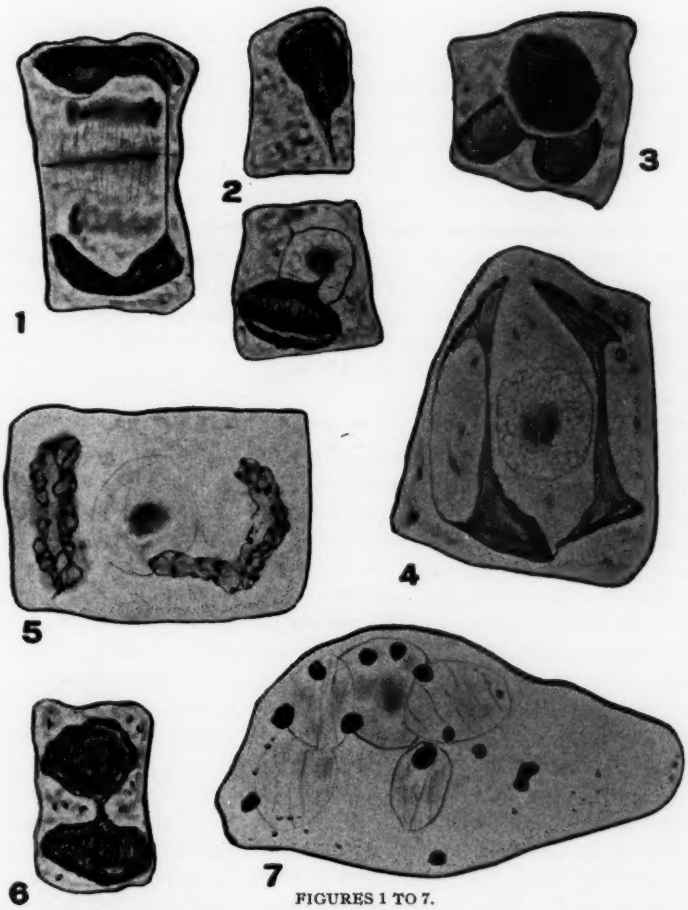
The plastonema is always well differentiated and distinct in outline. On the contrary the plastosome seems to be, sometimes at least, reduced in size. All aspects indicate that the plastonema is the more important substance, perhaps under certain conditions even absorbing the plastosome. This relation of a persistent "chromatic" substance is suggestive of the relation existing between the chromonema and the achromatic substance of the chromosomes.

The division of the plastid body has revealed some very interesting facts. This division is only the pinching in two of a body into two approximate halves and cannot be considered as a true bipartition in the same sense that one considers mitosis a division of the chromosomes. Some confusion may be avoided by calling the plastid division a cleavage.

Both plastonema and plastosome are always present and distinct at the time of cleavage. In general the plastonema divides before the plastosome, the latter being drawn out so that only a fine point of contact remains between the two halves of the dividing plastid (Fig. 3). These halves move apart so that at anaphase they are located at the poles of the dividing cell and are now connected by a very delicate filament (Fig. 1). Thus the two daughter cells will each contain a single plastid (Fig. 2).

At mitosis there seems to be only a general coöperation between the cleavage of the plastid and cell division. Usually the plastid divides only once between each two successive nuclear divisions. However, cases are not very rare which show four plastids in a single cell (Fig. 4). Only two plastid cleavages to one cell division could make this possible.

One feature too striking to pass unnoticed and which will perhaps appear of significance is the resemblance, both in form and in reactions to fixing reagents, of this sporogenetic plastid to the Golgi body. This similarity exists not alone between drawings but we can ascertain it from an actual



FIGURES 1 TO 7.

1.—Anaphase, archesporial tissue. Plastids at the poles of the cell. Champy-Kull fixation, Benda stain.

2.—Young cells soon after division; each one contains a single plastid, the plastosomes show the remnant of the filament. Da Fano.

3.—The horseshoe Golgi form taken by the plastid during cleavage. Champy-Kull fixation, Benda stain.

4.—Two plastids in the process of cleavage in the same archesporial cell. Kolatchev.

5.—The network structure of the plastid which strongly resembles the form of the Golgi body in gland cells. Kolatchev.

6.—The Golgi form of the plastid as observed in the androgones. Kolatchev.

7.—Somatic cell showing mitochondria clearly grading down to the limit of visibility. Kolatchev.

comparison of slides. Owing to the kindness of Dr. Jean Morelle and the late Professor Robert Bowen we had the privilege of studying animal preparations. The similarity between the two bodies falls naturally into three divisions. The first of these is that both forms are made up of two distinct parts, one being the chromatic ribbon which partially forms the border for the second, the achromatic interior substance. Secondly, the horseshoe form of the plastid (Figs. 2 and 3) is so similar in shape to the acroblast of Professor Bowen's preparations that the one might easily be confused with the other. Thirdly, the broken network structure of the Golgi spot of gland cells is occasionally observed (Fig. 5). Such a comparison between the plant plastid and the Golgi body of animal cells lends support to the opinion that the Golgi apparatus is truly a specialized cytoplasmic body. We shall return later to the meaning of the Golgi shape of the archesporial plastid.

II. *The Plastid during Spermatogenesis.*—During the androgonial divisions there is present in the spermatogenetic tissue a body which in all aspects is the same as the archesporial plastid (Fig. 6). We have now the double question of finding, first, the origin of this body, and second, its development during the transformation of the androcytes. The question is more difficult than that of the origin of the archesporial plastid because the latter comes from cells already containing plastids, while the antheridia originate directly from meristematic tissue containing no recognizable plastids. Antheridia of a two-cell stage reveal a number of bodies of varying sizes, the largest of which may develop into plastids. We find in cells cut off from the apical cell faint irregular areas of cytoplasm, while two or three cells away from the apical cell quite definite, though faint, young plastids may be seen.

The second question relates to the fate of this plastid during the period of transformation of the androcyte into the sperm. During this stage the Kolatchev and Regaud fixations are particularly rich in revealing well-defined bodies which have escaped the notice of previous investigators and which make this phase of development particularly difficult and interesting. Among these bodies there is one which appears to be derived from the androgonial plastid but in which the structure is very much changed. There is a possibility that it plays some part in the formation of the tail. It seems that Allen[2] mistook this structure for the nucleus. Much work remains to be done on this period of development—one which is rendered particularly difficult because of the scarcity of cell divisions and the fact that really good preparations are extremely hard to obtain.

III. *Mitochondria.*—As for the mitochondria, it was rather surprising to find them sometimes missing from archesporial cells (Figs. 1, 2 and 3) and almost always lacking in the androgones and always from the androcytes. This fact cannot be ascribed to faults in fixation or in staining.

first, because I have used standard mitochondrial techniques and second, because preparations which show no mitochondria in the archesporial cells, androgones or androcytes, give beautiful examples of these granules in somatic tissue.

Occasionally an archesporial line presents a series of cells grading from some with no mitochondria to other cells in which there are many. Moreover, when granules are present they most frequently grade down to the limit of visibility (Fig. 7). On the other hand, in our work up to this point no relation has been apparent between mitochondria and plastid. Consequently, although the work has by no means progressed sufficiently to warrant a definite statement concerning the mitochondria, it may be said that the evidence so far tends to lend itself to the interpretation of A. Meyer,[4] namely, that the mitochondria are a stage of development of a nutritive substance which at some particular period of formation happens to be visible in the cytoplasm.

IV. *Other Cytoplasmic Bodies.*—In addition to the plastid and the mitochrondria the cytoplasm contains other small bodies. Among the most frequently observed are the osmiophilic platelets already very well described by Bowen.[5] During the archesporial divisions their rôle seems to be rather passive. Whether or not they take an active part in meiosis or in spermatogenesis has not yet been determined. A certain variation of the Kolatchev technique brings out in the archesporial line structures strongly resembling a raspberry. There are, as well, short, irregular curved rods, intensely black after osmification, to which there is attached a small amount of a grayer substance. These bodies are, to say the least, puzzling and there is some doubt in the author's mind that our present cytological methods will be able to explain them.

V. *Vital Coloration.*—So far attempts to stain archesporial tissue and antheridia with neutral red have not succeeded. Whether or not this is due to the inability of the stain to penetrate into the living cell or to the absence of areas of protoplasm or vacuoles capable of taking the color is not known. Mature sperms do not stain, nor do weak solutions of the neutral red cause their whirling motion to cease.

In the case of young leaves we have been able to color only the torn edges, suggesting that in the bryophytes the neutral red stains only during or after death. This impression is also supported by the reaction of the cells of the paraphysis to the stain. Always cells which are clearly injured will stain. In only two preparations have I ever seen the network type of "vacuome" described by Guilliermond.[6] Quite frequently small neutral red staining droplets may be observed flowing together much as do droplets of oil on water. The impression derived from these experiments is that the neutral red causes a modification of the physical structure of the cytoplasm in the paraphysis cell. In the author's opinion

the resemblance between this neutral red "vacuome" and the animal Golgi apparatus is much more superficial than that existing between the plastid and the Golgi body.

So far my experiments do not conflict with the opinion of Guilliermond that plant vacuoles may be homologous with vacuoles in animal tissues, but those experiments which have been completed, together with those in progress, seem to indicate that the Golgi body of the animal cell, if it must have a plant homolog, may be found in the plastid, and that the Golgi body and the vacuome are two separate and distinct systems. This latter statement is analogous with the condition reported by Beams[7] for the pancreas.

VI. *Conclusions.*—We return now to the meaning of the special Golgi form assumed by the plastid during sporogenesis and probably during spermatogenesis. Emberger,[8] Mangenot[9] and Motte[10] have described the plastids as regressing into mitochondria in the critical stages of sporogenesis, spermatogenesis and of oogenesis. We have not as yet followed the fate of the plastid throughout the complete history of the formation of reproductive cells but it is evident that in the stages so far studied no regression has occurred nor has any definite relation between the plastid and the mitochondria been revealed. Regression supposes a diminution in the size of the plastid. In *Polytrichum* it not only grows from the size of any ordinary immature plastid to the size of an adult one, but, while doing so, it changes materially its form and chemistry to assume a definite specialized structure. Moreover, whatever the final fate of the plastid, its behavior during sporogenesis and spermatogenesis cannot be interpreted as the beginning of regression, for the regular division of this body presupposes a definite rôle during this stage. Whatever this rôle may be, it seems certain that the change in shape would not occur only for propagation by cleavage. For these reasons we are inclined to think that the Golgi form of the plastid is destined to play a special rôle in sporogenesis and in spermatogenesis, a rôle which can best be served by the Golgi form. All tests for starch in these tissues being negative, this rôle cannot be one of starch synthesis or storage. May it not, however, be concerned with nutrition, in a function somewhat similar to that played by the Golgi body, in the elaboration of some substance other than starch to be used by the cell itself or by neighboring cells?

* C. R. B. Fellow to Belgium, 1929–30.

** It is a pleasure to acknowledge my indebtedness to Professor Victor Grégoire for his many valuable suggestions and kindly interest throughout all of this work.

[1] Senjaninova, M., *Zeits. Zellforsch. Mikr. Anat.*, **6**, 1928 (464–492) (493–508).

[2] Allen, C. E., *Ann. Bot.*, **31**, 1917 (269–290).

[3] Owen and Bensley, *Am. J. Anat.*, **44**, 1929 (79–110).

[4] Meyer, A., *Ber. deutsch. bot. Gesellsch.*, **34**, 1916 (168–172).

[5] Bowen, R., *Zeits. Zellforsch Mikr. Anat.*, **6**, 1928 (689–725).

[6] Guilliermond, A., *Protoplasma*, **9**, 1930 (133–174).
[7] Beams, H. W., *Anat. Rec.*, **45**, 1930 (138–162).
[8] Emberger, L., *Arch. Morph. gén. expér.*, **1**, 1921 (1–184).
[9] Mangenot, G., *ibid.*, **9**, 1922 (1–340).
[10] Motte, J., *Ann. Sci. Nat.*, Series 10, **10**, 1928 (298–543).

THE NATURE OF THE PROSTHETIC GROUP IN LIMULUS HEMOCYANIN

(Preliminary Paper)

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At the suggestion of Professor A. C. Redfield we have undertaken a chemical investigation of the hemocyanin of *limulus polyphenus* with special reference to the nature of the prosthetic group. We are now reporting a few preliminary results which we have obtained, since considerable time must elapse before the next large quantity of horseshoe crab blood can be decomposed and the product purified.

The only previous work on the nature of the prosthetic group in hemocyanin is that of Philippi[1] who studied the hemocyanin of the snail. He made the important discovery that if the protein is decomposed with alkali a black insoluble material is formed which contains the copper of the original pigment. This material he believed to be analogous to the hematin formed from hemoglobin by a similar treatment; apparently the quantity of material at his disposal was too small to allow of a detailed examination since the copper content (7%), solubility and a strongly positive pyrrol test are the only facts reported.

We followed Philippi's procedure in the decomposition of the protein but employed the whole blood of the horseshoe crab without any attempt at a separation of the pure hemocyanin. Potassium hydroxide to the amount of 10 per cent was added to the blood and the reaction mixture was warmed to 40°–50° for 20–30 minutes at the end of which time the protein was largely in solution. After standing overnight, a greenish brown precipitate settled which was removed by centrifuging and washed with dilute potassium hydroxide and then water to remove the protein. The precipitate at this point contains, in addition to the copper complex of the prosthetic group, magnesium and calcium hydroxide and probably copper sulfide (and some protein unless the washing has been very complete). In working up one large lot of blood, the material was collected, dried and stored

in this form; the copper content was only 2.6 per cent. From 85 liters of blood 200 grams of such material was obtained; the copper content represented practically all the copper in the original blood (200 x 0.026 = 5.2 grams Cu, compared with 5.5 grams in the original 85 liters).

The copper complex may be separated from the other alkali-insoluble materials by first washing with 10 per cent acetic acid containing sodium chloride (to prevent the formation of a colloidal solution of the copper compound) and then dissolving the material in 50 per cent acetic acid, removing the insoluble copper sulphide and reprecipitating with alkali. A considerable quantity of the crude material referred to above was purified by essentially this procedure and was obtained as an amorphous black powder with the following composition: Cu = 21.5 per cent; N = 9.2 per cent; C = 39.5 per cent; H = 5.6 per cent; S = 8.0 per cent. Unfortunately the crude material appeared to have undergone considerable decomposition after standing six months and only a few grams of purified material were obtained; the dilute acid washings were bright green. In a series of experiments in which the final purification was carried out at once, the yield was about 320 milligrams per liter, and the acetic acid washings were colorless. The copper content of the product was 23 per cent which corresponded to the entire copper content of the whole blood.

The purified material is a black powder, insoluble in all the common neutral organic solvents and in aqueous acid or alkali. When freshly precipitated it dissolves readily in 50 per cent acetic acid and tends to pass into a colloidal solution in dilute acids in the absence of salts; after drying, the material is insoluble in all strengths of acetic acid. Although the copper complex is insoluble in aqueous alkali, it will dissolve in alkaline solutions if ammonia or some amine is present, apparently a soluble complex salt is found. Dark green solutions in aqueous ammonia and pyridine (with the addition of some sodium hydroxide) and a brown solution in dilute hydrazine hydrate are readily prepared with even the dried material. On acidification, the material is precipitated apparently unchanged. The use of ammonia or hydrazine hydrate is the best method of getting the dried material into solution. The green solutions apparently contain cupric copper, the brown, cuprous copper, since the latter may be formed by the addition of a reducing agent; the transformation back and forth by means of sodium hydrosulphite and hydrogen peroxide is rapid but attended by some decomposition. The absorption spectra of the green and brown solutions consist of only one broad band in the blue which extends into the extreme violet.

The copper may be removed from the complex in acid or alkaline solution. If the dark green solution in 50 per cent acetic acid is treated with an equal volume of concentrated hydrochloric acid and warmed, the color disappears, the copper passes into the ionic condition, and hydrogen sulphide

is evolved; at least two organic products are formed, one an amphoteric substance insoluble at the neutral point and the other a material soluble in water which has not been obtained in a crystalline form. The removal of the metal in alkaline solution appears to take place with less drastic decomposition of the whole molecule. If a solution of the pigment in ammonia is allowed to stand in the air with frequent shaking for several days, the color almost vanishes; on cautious acidification with acetic acid, an amphoteric solid is precipitated. The filtrate contains ionic copper but no sulphide; after removal of the copper, as copper sulphide, and evaporation to dryness, a residue is obtained amounting to about one-third of the material while the amphoteric precipitate amounts to about two thirds.

The amphoteric precipitates from both the acid and alkaline decomposition contain nitrogen and sulphur and appear to be similar. The yield from the acid decomposition is very small, however. The amphoteric material is slightly soluble in hot alcohol. The nitroprusside, Molisch, biuret, and ninhydrin tests were negative; the material does not reduce Fehling's solution. Boiling with moderately concentrated hydrochloric acid does not render the material water-soluble in neutral solution, although when the copper complex is decomposed with hydrochloric acid, the bulk of the product is water soluble. However, since the sulphur linkage is also attacked under these conditions (hydrogen sulfide is evolved), it is clear that the decomposition in this case involves more than the mere removal of the copper.

From the evidence now available it would appear that the black copper-containing material is a complex salt of an amino acid which contains sulphur; on the basis of the tests and properties just described it is extremely unlikely that we are dealing with any of the known amino acids.

Unlike Philippi's product from snail hemocyanin, our material did not give any pyrrol test with a pine splint or with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde). These tests were uniformly negative on the crude and purified material and on the products obtained after the removal of copper. However, on fusion with potassium hydroxide, both the copper-containing pigment and the copper-free amphoteric material yielded a volatile substance which gave a strong positive test with the pine splint and Ehrlich's reagent. In the hope of obtaining volatile pyrrols by reduction, the pigment was treated with hydriodic acid and phosphonium iodide under conditions which yield large amounts of volatile pyrrols from hematin; no trace of pyrrols could be detected either from the crude or purified material. Thus, if pyrrol rings are present in our material they must be carboxylated; however, since the tests in question are also given by indole derivatives, and since decomposition with fused potash is very drastic it would be unwarrantable to conclude that the compound contains a pyrrol ring. The absence of an intense color or characteristic spectrum after

the removal of the copper, as well as the negative results obtained in the hydriodic acid reduction prove that the prosthetic group is neither a porphyrin nor a closely related substance such as a bile pigment.

The stability of the black copper complex and its tendency to form soluble, highly colored complexes with amines (and also denatured albumen) indicates that the compound we have in hand is involved in the union of the copper to the protein in hemocyanin. The function of the prosthetic group thus appears to be the same as that of protoporphyrin in hemoglobin, namely, to provide a basis for a very stable metallic complex. Beyond this analogy, however, there seems to be little or no chemical relationship between the prosthetic groups in limulus hemocyanin and hemoglobin. It should be noted that since limulus hemocyanin differs markedly in its copper content from the hemocyanin of other species,[2] the conclusions we have drawn do not necessarily apply to the nature of the prosthetic group in the other hemocyanins.

We are greatly indebted to Mr. I. S. Danielson for his assistance in procuring the initial material and for carrying through a number of the experiments.

[1] E. Philippi, *Zeit. Physiol. Chemie*, **104**, 88 (1919).

[2] Redfield, Coolidge and Shatts, *J. Biol. Chem.* **76**, 185 (1928).

THE PHOTOCHEMICAL DISSOCIATION OF NITROGEN PEROXIDE

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When the substance, nitrogen peroxide, consisting of a mixture of two gases, nitrogen peroxide, NO_2, and nitrogen tetraoxide, N_2O_4, is illuminated with approximately monochromatic light from a quartz mercury arc, an increase in pressure occurs which is greater than that to be expected from the heating effect of the radiation on the gas. The abnormal rise in pressure has been attributed to photochemical decomposition. Norrish[1] almost exclusively studied the equilibrium involved, and in later work[2] investigated the quantum yield so far as to determine the number of oxygen molecules formed in the reaction system per quantum of light absorbed. One molecule of oxygen was produced for every quantum absorbed in the spectral regions 2700–3160 Å and 3660 Å, while at longer wave lengths of 4050 Å and 4360 Å, 0.37 and 0.0 molecules of oxygen, respec-

tively, were produced for each quantum absorbed. Dickinson and Baxter[3] effectively showed the products of the photochemical decomposition to be nitric oxide and oxygen. The variation of the quantum yield with wave lengths of light used in illumination leads to the belief in a photochemical threshold which must exist between 4050 Å, the region in which dissociation began to take place, and 3660 Å, the region where full photochemical equivalent was observed. The quantum yield at intermediate wave lengths has not been investigated.

Since nitrogen peroxide contains two kinds of molecules, the analysis and formulation of the reaction becomes increasingly difficult. For instance, it was equally as possible to begin upon the assumption that the photoactive species was either the NO_2 molecule or the N_2O_4 molecule. On the other hand, it was not inconceivable that both molecules may be involved. The question can at least be partially solved by a study and

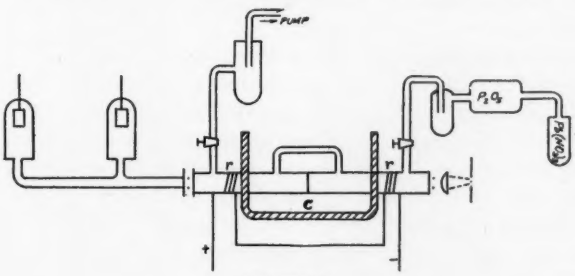

FIGURE 1

review of the existing absorption spectrum data concerning these two gases.

Harris[4] reported the absorption spectrum of nitrogen peroxide to consist of two separate and distinct types of absorption. The first, which was attributed to NO_2, extends from the visible region of the spectrum into the ultra-violet as far as 2250 Å, and exhibits a complex banded structure. The second, which is attributed to N_2O_4, begins within the limit of experimental error, at about 4000 Å, and exhibits a *continuous* absorption spectrum which increases in intensity to a maximum at about 3500 Å. These results were confirmed in hitherto unpublished work of the writer. While photographing the absorption spectrum of nitrogen pentoxide, the writer found it necessary to photograph both the absorption spectrum of nitrogen peroxide and nitrogen tetraoxide, since the latter two gases were ultimately produced in the decomposition of the pentoxide, and the presence of either in appreciable amounts would have masked the true absorption spectrum of the pentoxide. Photographs of NO_2 at the temperature of 140° C. and solid N_2O_4 at the temperature of liquid air were taken, the following precautions being observed:

Pure nitrogen peroxide was made from heated lead nitrate, passed over phosphorus pentoxide, and condensed into a trap surrounded by carbon dioxide–ether mush. The trap, the 30-cm. length quartz absorption cell, the drying tube and the lead nitrate bulb were pumped free of air, and the inlet and outlet stopcocks to the absorption cell were closed. By taking the freezing mixture away from the trap, and manipulating the inlet stopcock, any desired quantity of the brown gas could be admitted to the absorption cell. The absorption cell was constructed with two compartments such that light traversing the cell had to pass through three quartz windows. The gas was free to flow from one compartment to the other by way of the by-pass around the inner window. Figure 1 illustrates the apparatus used. The source of continuous ultra-violet light was afforded by a hydrogen discharge tube. The light rays from the discharge tube, after passing the length of the absorption cells, were focused on the slit of the spectrograph. A Hilger E-37 quartz spectrograph was used in addition to a larger E-3 instrument.

A number of successive exposures were taken with NO_2 gas in the absorption cell, at varying pressures less than atmospheric. The temperature was kept at 140° by means of electrical heating elements which were wrapped the whole length of the absorption cell, and this temperature was measured by means of a thermometer enclosed within the asbestos casing about the cell. These exposures are shown upon plate 1. The complex banded absorption attributed to NO_2 by Harris begins in the visible region of the spectrum, rises to a maximum at approximately 3900 Å, and then decreases to a minimum intensity at approximately 3200 Å. Apparently, NO_2 absorbs radiation very weakly in the region 3200 to 2495 Å. A second series of bands begin at 2490 Å, the wave lengths of which have been previously determined by Harris, for the reason that they possess a very much simplified appearance in comparison to those bands due to NO_2 in the visible spectrum.

Plate 1 also shows a photograph made with solid N_2O_4. The following method was used to obtain this photograph. Referring again to figure 1 the gaseous contents of the absorption cell, after photographing the mixture of the two oxides at room temperature, were condensed out upon the inner window, by pouring liquid air into the plaster-of-paris container *C*, which was moulded about the absorption cell. The brown gas, which turned into white crystalline N_2O_4 upon the inside window, therefore, formed a semi-transparent layer on both sides of the quartz disk. This was ascertained by viewing the absorption cell, end on, with the eye while the hydrogen discharge tube was turned on. The ends of the absorption cell which protruded from the cold container *C* were kept free from condensed moisture, by means of the electrical heating elements, *r*. This photograph, therefore, shows the region of the spectrum absorbed by

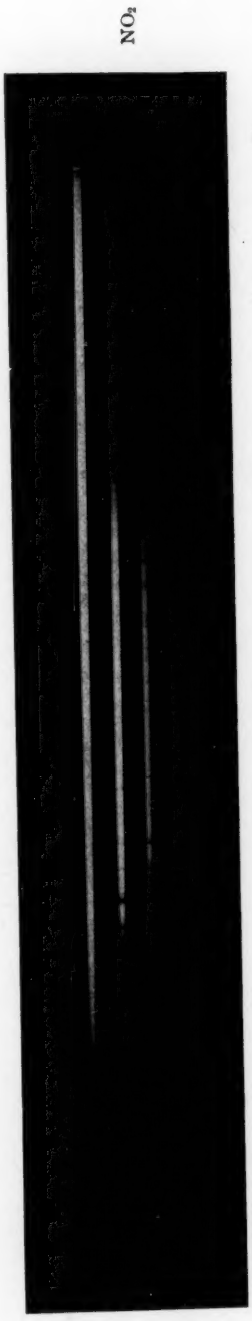

PLATE 1.

crystalline N_2O_4 at the temperature of liquid air, $-182°C$. Absorption begins, within the limit of experimental error, at about 3820 Å, increasing in intensity toward the shorter wave lengths. Some diffuse light was transmitted below 3820 Å, but this was to be expected, and may be accounted for in reflection of light from crystal face to crystal face in the thin layer. This photograph is doubtless of no other value except that it enables the long wave length limit of absorption to be determined for N_2O_4 while in the crystalline state.

In general, within the limit of experimental error, the light energy of activation should not vary to a great extent, regardless of the state, solid, liquid, or gas, which the molecules might be in. This is not, however, exactly correct. The heat of activation in the gaseous state, according to conventional thermochemical calculations, differs from the heat of activation in the solid state, by an amount of the order of the difference in thermal energy possessed by the substance at two temperatures. The difference in thermal energy in this case may be divided in five separate parts, $\Delta E_1 = \int_{T_1}^{T_2} C_p dT$, the energy necessary to raise a mol of N_2O_4 from the temperature of liquid air to its melting point, $-10°C.$, plus the heat of fusion, ΔE_2, plus $\Delta E_3 = \int_{T_2}^{T_3} C_p dT$, the energy necessary to raise the substance from its melting point to its boiling af 18°C., plus the heat of vaporization, ΔE_4, plus $\Delta E_5 = \int_{T_3}^{T_4} C_p dT$, the energy necessary to raise the gas from its boiling point to room temperature. The specific heat of nitrogen tetraoxide is not known, and for that reason, assumed values must be used. Making use of Kopp's Law, C_p for solid N_2O_4 is about 22 calories per mol. The first quantity above then becomes $\Delta E_1 = 3.984$ kilo-calories. ΔE_2 is 0.034 kilo-calories (I. C. T. value). ΔE_3 becomes 0.670 kilo-calories, assuming the rough value of 24 calories per mol for the heat capacity of liquid N_2O_4. ΔE_4 is probably very small. The specific heat of gaseous N_2O_4 is doubtlessly less than for two mols of NO_2 and is not greater than about 18 calories per mol. ΔE_5 then becomes 0.126 kilo-calories, and the total change in thermal energy between the temperature of liquid air and the room temperature of 25°C., is of the order of 4–5 kilo-calories. The long wave length limit observed for N_2O_4 at liquid air temperature is 3820 Å corresponding to 74.6 kilo-calories for the least heat of optical activation to which the molecule is susceptible. Subtracting this value of about 4.5 kilo-calories energy, which must be present in the gas at 25°C. from the heat of activation at $-182°C.$, 70.2 kilo-calories is obtained which must correspond to the least energy of optical activation at the higher temperature. This latter

figure for N_2O_4 at room temperature corresponds to wave lengths of light at 4060 Å.

This method of treatment may account for the shifting of the absorption spectrum of N_2O_4 toward the longer wave lengths with increasing temperature. Harris shows the absorption spectrum of liquid N_2O_4 to begin at about 4000 Å.

The majority of photochemical reactions are very complicated, involving transfers of energy so rapid as to obscure the original primary process. Only those changes which find themselves within the boundary of Einstein Photochemical Equivalence Law are capable of analysis, and even then these become difficult for the study of complex molecules. The problem involving the correlation between the thermal energy required to decompose a substance, and the optical energy necessary to bring about the same transformation has been partially understood in the study of the simpler types of molecules during the last few years. Franck[5] was able to conclude that Cl_2, I_2 and Br_2 dissociated into atoms beyond a certain region in their absorption spectra. This region was the convergence limit of the band series, beyond which continuous absorption and complete primary dissociation occurred. The energy of dissociation for the thermal change was somewhat less than the energy involved in the photochemical dissociation, but the difference was accounted for in assuming that one of the atoms on dissociation was left in the 2^2P_1 excited state. For simple diatomic molecules (H_2, I_2, HI, HCl, O_2, etc.) the linkages or bonds holding the atoms together were classed as polar or non-polar. Franck, Kuhn, and Rollefson[6] showed the energy calculated from the long wave limit of the continuous absorption for polar molecules to be equal to the thermal energy of dissociation. This is the case for the alkali halides. The non-polar molecules gave an energy of dissociation corresponding to the long wave-length limit of continuous absorption greater than the thermal energy of dissociation, which was equivalent to the thermal energy of dissociation plus an amount of energy necessary to excite one of the atoms. The halogen molecules were examples of this latter type.[7]

These observations have been limited to diatomic molecules. With more complex molecules, in the special case where a region of continuous absorption indicated an electronic activation of some linkage in the molecule, the dissociation process was considered analogous to the cases of photochemical dissociation in diatomic molecules, and since the study was not confined to band structure with quantized states of molecules, but only to continuous spectra, it became difficult to draw a distinction between the primary processes taking place in the two instances (diatomic molecules and polyatomic molecules). Iredale and Wallace[8] measured the long wave-length limit of the continuous absorption due to the com-

plex organic molecules, methyl iodide, ethyl iodide, iodo-benzene, etc., and obtained an unmistakable agreement between the thermal energy of dissociation and the optical energy of dissociation. Urey, Dawsey, and Rice[9] considered the continuous absorption spectrum of nitrogen pentoxide to indicate a primary dissociation of the molecule.

If the continuous character of the nitrogen tetraoxide spectrum may be interpreted to indicate a primary photochemical dissociation for that molecule, the difficulty which prevents a clear understanding of the photochemical decomposition in nitrogen peroxide–tetraoxide mixtures and the quantum yield obtained therein is considerably altered. Measurable quantum yields were obtained only over the spectral range covered by the continuous absorption spectrum of N_2O_4. The photochemical decomposition may be complicated by secondary reactions in the presence of nitrogen peroxide molecules, the tendency being to obscure, rather than assist, the principal photochemical change while on the other hand NO_2 may act as a photo-catalyst, i.e., an energy carrier, whereby some such mechanism as, first, absorption of light by NO_2, and second, a collision between the activated NO_2 molecule and an N_2O_4 molecule, resulting in the disruption of the N_2O_4, may occur. Aside from this possibility, the absorption spectra of these two gases indicate that only one main, primary photochemical process is likely to take place in a mixture of the two. The products of the decomposition are known to be nitric oxide and oxygen, the absorption spectra point to N_2O_4 as being the photoactive constituent, and the thermal data offers a source from which to check energy relations involved in the photochemical change. The primary photochemical reaction is therefore thought to be

$$N_2O_4 \xrightarrow{h\nu} 2NO + O_2(^1S) \qquad (1)$$

where $h\nu = \frac{74.6}{N}$ kilo-calories corresponding to the wave length 3820 Å. The corresponding energy for the thermal change is 39.6 kilo-calories. It is assumed that the oxygen molecule is left in the ^1S excited state on dissociation of the nitrogen tetraoxide molecule. The energy necessary to excite an oxygen molecule in the normal ^3S to the ^1S excited state is 1.62 volts,[10] and is equivalent to 35 kilo-calories. Thus, the sum of the energy for the thermal change plus the energy of excitation of the oxygen molecule is 74.6 kilo-calories, and agrees very well with the energy value obtained from the short wave-length limit of the continuous absorption spectrum.

Summary.—1. The absorption spectrum of nitrogen dioxide and nitrogen tetraoxide have been photographed at room temperature and the temperature of liquid air, respectively, and compared with previous work.

2. Consideration of the absorption spectra of these two gases leads to the conclusion that the primary photochemical decomposition in nitrogen peroxide, which consists of nitrogen peroxide and nitrogen tetraoxide, is due to the tetraoxide constituent.

3. The threshold for complete photochemical equivalence is calculated to be at about 3800 Å.

4. The mechanism, $N_2O_4 \xrightarrow{h\nu} 2NO + O_2(^1S)$, is proposed to account for the primary photochemical dissociation, and the continuous absorption spectrum of N_2O_4. The energy changes involved agree closely with thermal data.

[1] Norrish, *J. Chem. Soc.*, Part 1, 761 (1927).
[2] *Ibid.*, Part 2, 1158 (1929).
[3] Dickinson and Baxter, *J. Am. Chem. Soc.*, **50**, 774 (1928).
[4] Harris, *Proc. Nat. Acad. Sci.*, **14**, 690 (1928).
[5] Franck, *Trans. Farad. Soc.*, **21**, 536 (1926).
[6] Franck, Kuhn and Rollefson, *Zeit. Phys.*, **43**, 155 (1927).
[7] Franck and Kuhn, *Zeit. Phys.*, **43**, 164 (1927).
[8] Iredale and Wallace, *Phil. Mag.*, **8**, 1093 (1929).
[9] Urey, Dawsey and Rice, *J. Am. Chem. Soc.*, **51**, 3190 (1929).
[10] Mulliken, *Phys. Rev.*, **32**, 880 (1928).

THE STUDY OF ABSORPTION SPECTRA OF ORGANIC COMPOUNDS AT LIQUID AIR TEMPERATURES

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It is well known that certain broad bands in the ultra-violet absorption spectrum of benzene are broken into finer lines at liquid air temperatures. At the suggestion of Professor Linus Pauling we have examined the visible absorption spectra at low temperatures of solutions of porphyrins and certain other colored organic compounds to see if the same phenomenon could be noted. As Professor Pauling has suggested, the characteristic spectrum of a porphyrin, which consists of a number of relatively very narrow bands, is probably due to the fact that the chromophoric groups are protected from thermal disturbances by the large cyclic system; the porphyrins might thus be considered analogous to the rare earths. It might be expected, therefore, that the visible spectrum of a porphyrin would be resolved into narrower bands at low temperatures. The sub-

stances we have studied are protoporphyrin dimethyl ester, phylloporphyrin, chlorin e, bilirubin and pinacyanole. All these substances have strong absorption bands in the visible. Chlorin e and bilirubin like the porphyrins contain pyrrol rings; the relation of the former to the porphyrin is still unsettled, the latter almost certainly does not contain the characteristic porphyrin ring. The photosensitizing dye pinacyanole was chosen because of its relatively narrow band.

Dilute solutions of the materials in alcohol and ether were examined both visually and photographically with a smaller Hilger constant deviation spectrometer. In order to keep the substance at the temperature of liquid air, the solution was placed in a thin walled test tube and congealed by immersion in liquid air in an unsilvered Dewar flask. The test tube was then raised so that about 1 cm. of the gel projected above the surface of the liquid air and the beam of light was passed through this exposed layer. With care, crystallization of the solvent usually could be avoided.

FIGURE 1

Absorption spectrum of a porphyrin at room temperature and in liquid air. (A = room temperature, B and C at liquid air temperature, C longer exposure than B. 10 mg. of protoporphyrin dimethyl ester in 1 liter of ether.)

As judged by visual observation, the widest bands in the porphyrins were broken into fairly uniformly spaced narrow bands. The other substances failed to show this phenomenon. Photographs of the porphyrin spectra at room temperature and at liquid air temperatures brought out the effect even more clearly. (Fig. 1.)

The positions of the narrow bands obtained at low temperatures with protoporphyrin dimethyl ester, were measured by comparison with an iron arc. The narrower band with a center near λ 53,630, broke up into three components of frequency 18,462, 18,648, 18,909 cms.$^{-1}$. The wider band (center at approx. λ 5000) yielded six components: 19,488, 19,668, 19,887, 20,107, 20,328, 20,544 cms.$^{-1}$. It is interesting that the frequency differences in the first group are 186 and 260 cms.$^{-1}$ and in the second 180, 219, 220, 221, 226 cms.$^{-1}$. This suggests that the components in each group represent vibrational bands having a common value for the *change* in the vibrational quantum number.

It seems probable that the phenomenon of resolution of bands at low temperatures may be characteristic of compounds having certain cyclic systems. If this is the case a spectroscopic study of solutions of compounds at liquid air temperatures may serve as a ready means of recognizing the presence of such characteristic cyclic systems as are present in the porphyrins. In particular we hope that a differentiation between the porphyrins and the closely related chlorophyll derivatives may be possible. A very large number of these compounds have been prepared in this laboratory in a high state of purity and their spectra at low temperatures will be examined in the near future.

A CORRECTION

Due to a mistake in the preparation of Table VII, p. 134, in our article on "The Decomposition of Nitrogen Pentoxide at Low Pressures" in these PROCEEDINGS, **16,** 129–135 (1930), the time which is given in the first column is incorrect. The values given should each be multiplied by 2.3, the conversion factor for changing from the natural to Brigg's logarithms. The constants given in the Table are correct, because they were calculated with the correct time.

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